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PLACENTAL GROWTH FACTOR LEVELS IN POPULATIONS WITH HIGH VERSUS LOW RISK FOR CARDIAC DISEASE AND STRESSFUL PHYSIOLOGICAL ENVIRONMENTS SUCH AS MICROGRAVITY: A PILOT STUDY

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Running Title: Placental growth factor levels in cardiac disease

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ABSTRACT

This pilot study compared placental growth factor (PlGf) levels in populations with high versus low risk for cardiac disease. Previous experiments from our laboratory (Sundaresan *et al.* 2005; 2009) revealed that the angiogenic factor PlGf was up-regulated in modeled microgravity conditions in human lymphocytes leading to possible atherogenesis and pathogenesis in microgravity. Since the findings came from simulated microgravity experiments, there is a strong link to its usefulness in the microgravity field as a biomarker. The relevance is enhanced because in life on earth, it is a cardiovascular inflammatory marker. Studies on the levels of PIGF would help to give a hint about the risk of heart failures in astronauts.

The investigations here were only to confirm that in a cardiovascular stressed population such as CAD and ACS patients, PlGf could be overexpressed.
We desired to evaluate this marker in stressed cardiac disease patients. PlGf is a marker of inflammation and a predictor of short-term and long-term adverse outcome in acute coronary syndrome (ACS). In addition, elevated PlGf levels may be associated with increased risk for coronary heart disease. PlGf levels were determined in thirty-one patients undergoing cardiac catheterization for reasons

other than ACS and in thirty-three low-risk asymptomatic subjects. Additional data on traditional cardiac risk factors for both populations were also compiled and compared. We found that PlGf levels were significantly higher in the high-risk than low-risk population and correlated inversely with HDL-cholesterol but directly with the triglyceride levels. With further validation, PlGf may prove a useful addition to the armamentarium of noninvasive biomarkers for cardiac disease including a new area of stressful physiological conditions such as microgravity.

Key words: PlGf; catheterization; coronary artery disease, ACS

INTRODUCTION

Previous experiments from our laboratory (Sundaresan *et al.* 2005; 2009) revealed that the angiogenic factor PlGf was up-regulated in modeled microgravity conditions in human lymphocytes. Prior to this both in true space flight and modeled microgravity culture conditions in our laboratory and in others, immune suppression was observed via depressed lymphocyte activation and locomotion (Sundaresan *et al.* 2004). Delineation of the mechanisms via signal transduction revealed mishaps in trans-membrane signaling at or above the level of Protein Kinase C (PKC). This led to further characterization of the adaptational versus functional alterations in the human lymphocytes in response to microgravity. The next step in the PlGf

experiments was to corroborate the gene array findings with real time PCR. RT PCR was carried out for PlGf to confirm the gene array results (Sundaresan *et al.* 2005; 2009). Peripheral blood lymphocyte cells from normal human donors were isolated by the Ficoll-Paque method. Half the cell population obtained was split into two for 1g and modeled microgravity culture (Sundaresan *et al.* 2002, 2004). Cells were harvested at 72 hours and pelleted for RNA extraction and Protein determination by standard protocols. RT- PCR was performed with hPlGf and hGAPdH primers by standard procedures starting with 300 ng of RNA per sample in triplicate.

From the RT- PCR conducted on peripheral blood from four normal donors all of whom were male, previous work (Sundaresan *et al.* 2005; 2009) showing up-regulation of PlGf was confirmed. The data from our laboratory and from other laboratories (Heeschen *et al.* 2004; Sundaresan *et al.* 2004) suggests that microgravity can lead to increased inflammatory responses. The implication of this data cannot be ignored especially as missions to the Moon and Mars will soon be reality.

Anecdotal evidence from astronaut samples (serum or urine, baseline, pre and post flight) will be proposed soon. A holistic approach from cell to whole body physiology especially looking at important physiological effectors is warranted. Hence we hypothesized that PlGf would be elevated, compared to in healthy

asymptomatic subjects, in stable patients who were nonetheless still at high risk for cardiac disease.

Placental induced growth factor (PlGf), first detected in the placenta, is a member of the vascular endothelial growth factor family (VEGF). PlGf is strongly up-regulated in early and advanced atherosclerotic lesions and acts as a primary inflammatory instigator of atherosclerotic plaque instability (Lenderink et al 2006). Results from the CAPTURE trial suggest that PlGf is an independent biomarker of short-term adverse outcome in patients with ACS (Lenderink et al 2006). There are also data suggesting that elevated PlGf is associated with adverse long-term outcome post ACS (Lenderink *et al* 2006). A nested-case control investigation of the Nurses Health Study, a 14 year follow-up of 32,826 women who were healthy at baseline, showed elevated PlGf as a predictor for coronary artery disease. Previous investigations revealed a more than five-fold increase ($p<0.001$) in angiogenesis inducers, including PlGf, in normal human lymphocytes in a physiological stress environment in modeled microgravity, a space cell culture analog. Up-regulation of PlGf suggests de-regulation of cardiovascular signaling pathways (Cassidy *et al* 2009). These observations raise the question of PlGf participation in the stresses of cardiovascular disease in different altered physiological environments such as spaceflight. Genetic response suites in human

lymphocytes in response to microgravity and high altitude stress enable identification and further study in order to augment human physiological adaptation to novel environments.

The DNA micro array has the potential to identify novel genes involved in mediating adaptation to environments associated with stress. The study of such genes essential to adaptation is valuable for identifying potential new targets for therapeutic countermeasures, or as predictive biomarkers of novel response. PlGf is named for the organ in which it was first detected and is a newly described molecular marker of inflammation.

It is a major component in the inflammatory process and a proven marker for event risk in the context of acute coronary syndrome (ACS), stroke and other cardiovascular conditions (Heeschen *et al* 2004). It is now considered a more specific biomarker than C-reactive protein for predicting stroke and myocardial infarction, and is up-regulated significantly in early onset and progressive stages of cardiovascular dysfunction. It may be an early indicator of ACS in individuals who suffer chest pain serious enough to bring them to emergency rooms, with elevated levels of PlGf also predicting increased risk of ACS-related mortality (Heeschen *et al* 2004). Previous experiments from our laboratory (Sundaresan *et al.* 2005; 2009) revealed that the angiogenic factor PlGf was up-regulated in modeled microgravity conditions in human

lymphocytes. We thus hypothesized that PlGf levels would also be elevated, compared to in healthy asymptomatic subjects, in stable patients who were nonetheless still at high risk for cardiac disease. Since the findings came from simulated microgravity experiments, there is a strong link to its usefulness in the microgravity field as a biomarker. The relevance is enhanced because in life on earth, it is a cardiovascular inflammatory marker. Studies on the levels of PIGF would help to give a hint about the risk of heart failures in astronauts.

The investigations here were only to confirm that in a cardiovascular stressed population such as CAD and ACS patients, PlGf could be overexpressed.

METHODS

After obtaining institutional review board approvals for the pilot study and written informed consent from each participant, blood samples were taken just before catheterization from 31 patients undergoing cardiac catheterization and from 33 healthy controls. PlGf levels were measured in all blood samples by an immunoassay (R and D, Minnesota, DPG000).

Data related to traditional cardiac risk factors were also collected (Table 1), including total cholesterol, LDL, HDL and triglyceride levels (Table 2). Results of cardiac catheterization and of other studies such as EKG, echocardiogram and cardiac stress testing were also compiled.

RESULTS

PlGf in the asymptomatic subjects studied thus far (N=33) is just 16.5 ± 6.6 ng/L (mean ± SD) versus 91.2 ± 51.6 ng/L ($p<0.001$) in a subgroup of patients with known coronary artery disease (N=30). These groups were all patients who went in for catheterization and most had proven CAD. In the latter group, the mean value is increased well beyond the clinical threshold level (>27ng/L) (Table 2, Figure 1). Case history details were also collected to analyze correlations between PlGf and cardiovascular risk markers. There was an inverse correlation between PlGf and HDL cholesterol (Figure 2) and a direct correlation between PlGf and triglycerides (Figure 3).

DISCUSSION

In this pilot study, patients undergoing cardiac catheterization for any reason had PlGf levels significantly higher than healthy control subjects. And in turn our small group of healthy controls had PlGf levels similar to those of the healthy women included in the 32,826 Nurses Health Study (PlGf = 16.1) (Cassidy *et al* 2009). Our patients undergoing catheterization were older, more predominantly male and had more cardiac risk factors than the healthy controls. While none of the catheterized patients was undergoing catheterization for evaluation of possible ACS, five (16%) had a personal history of a prior myocardial infarction, ten more (32%) had significant coronary artery disease (CAD) discovered during the cardiac catheterization that required intervention, and a total of 24 (77%) had some form of known previous or current CAD. There is no question therefore that the catheterized patient population had a high burden of cardiac disease, particularly CAD.

PlGf was inversely proportional to HDL-cholesterol level and directly proportional to triglyceride level. This may signify a true correlation or simply the fact that all three of these biomarkers may be construed as risk markers for CAD. Being a pilot study, our study is insufficiently powered to explore any but the most superficial of relationships. However, it is worthwhile to note that other studies have shown similar correlations in larger populations (Cassidy *et al* 2009).

Lastly, LDL-cholesterol did not appear to be different amongst the two groups (Figure 4). This may be due to a number of reasons including power, but also the fact that a larger number of patients in the catheterization group were on lipid lowering medications that primarily act on LDL.

In patients with ACS, the presence of thrombogenic contents in the circulation may be responsible for the plaque rupture (Cassidy *et al* 2009). Specifically, most cases of ACS probably result from platelet activation and thrombus formation (Cassidy *et al* 2009). In previous studies, levels of PlGf have not correlated with levels of troponin (Cassidy *et al* 2009), the latter being principally a marker of cardiac muscle injury. These results suggest that PlGF elevations are likely driven by a different mechanism of cardiovascular injury, *i.e.,* inflammation and atherosclerosis. Overall, the present findings suggest that the concentration of PlGF, a more specific marker of vascular inflammation, is likely to be significantly increased in populations with high cardiac disease burdens.

Besides the overall small number of participants, this pilot study was also limited by the heterogeneity of cardiac disease types in the catheterized population (i.e., not just CAD) and by the significant age, gender and risk factor differences between the catheterized and healthy control groups (Tables 1 and 2). Nevertheless there seems to be little question that patient groups with a high burden of coronary heart disease have higher levels of PlGf.

Moreover our results are consistent with those of previous studies (Cassidy *et al* 2009). Hence in conclusion, the elevated levels of PlGf at the protein and RNA levels both in *in vitro* and *in vivo* models of analog microgravity suggest that further studies of PlGf in astronauts will be beneficial to the space program. This pilot study was to ascertain if PlGf was elevated in high risk cardiac disease populations compared to healthy controls to pave the way for future studies in astronauts and in aviation medicine.

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FIGURE LEGENDS

Figure 1. PlGf levels in control subjects (N=33; 16.5 ± 6.6 ng/L, mean ± SD) versus in a (subgroup of patients with known coronary artery disease (N=30; 91.2 ± 51.6 ng/L ($p<0.001$)).

Figure 2. Inverse correlation between levels of PlGF and high density lipoprotein (HDL)

Figure 3. Direct correlation between levels of PlGF and triglycerides in the entire group of catheterized patients.

Figure 4. Lack of correlation between levels of PlGf and low density lipoprotein (LDL) in the entire group of catheterized patients

Table 1. Demographics

	Catheterized Patients	Controls
Age	64.4	49.5
Male	97%	67%
Coronary Artery Disease	77%	none
Traditional Cardiac Risk Factors and related	3.97	1.5
Hypertension	87%	26%
Hyperlipidemia	77%	37%
Lipid Lowering Agent	67%	17%
Hyperglycemia or Diabetes	48%	22%
Tobacco Use	48%	none

Table 2. Results

PlGf and Fasting Lipid Panel			
	Catheterized Patients	Controls	*p*
PlGf	91.2	14	p<0.001
Cholesterol	162.6	173.9	p<0.1
LDL	94	104.1	P<0.2
HDL	36.8	53.9	p<0.001
Triglycerides	149.4	100.9	p<0.001

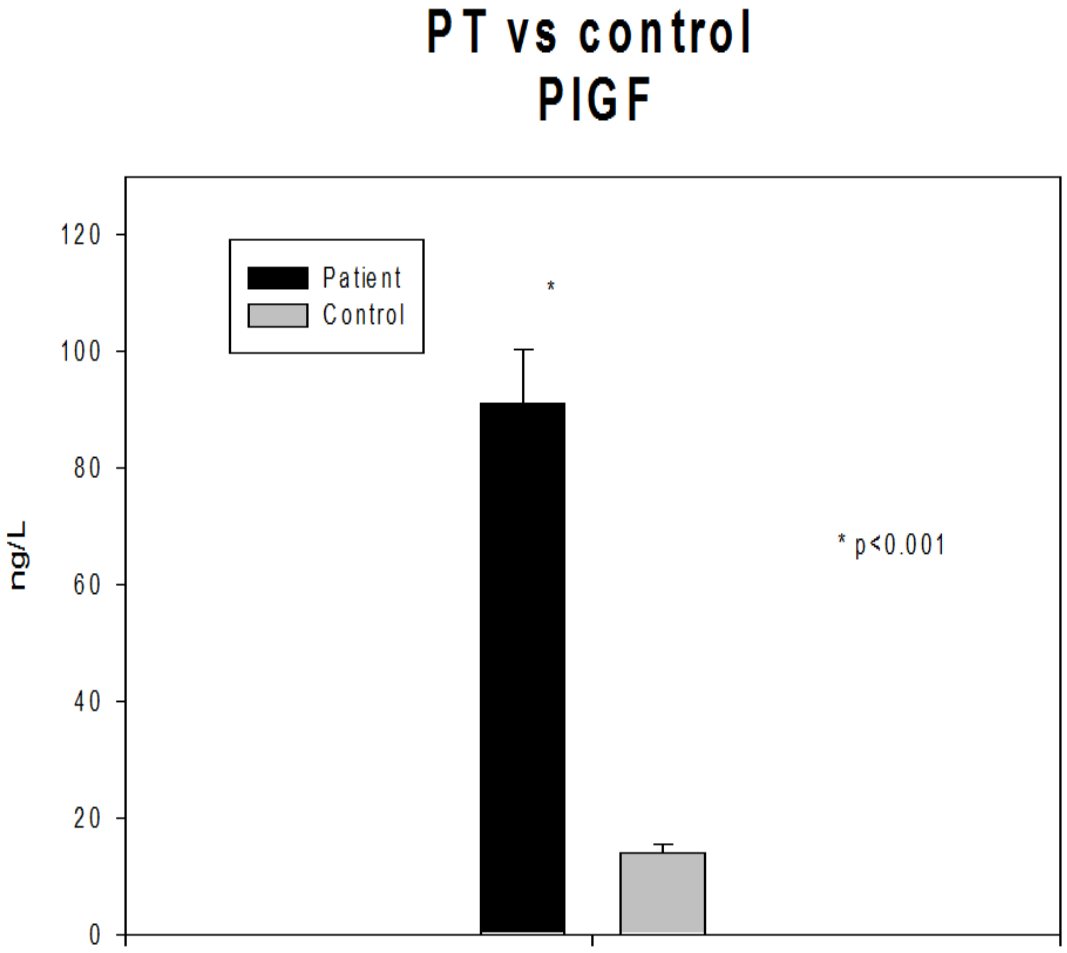

Figure 1. PlGf levels in catheterized patients versus controls

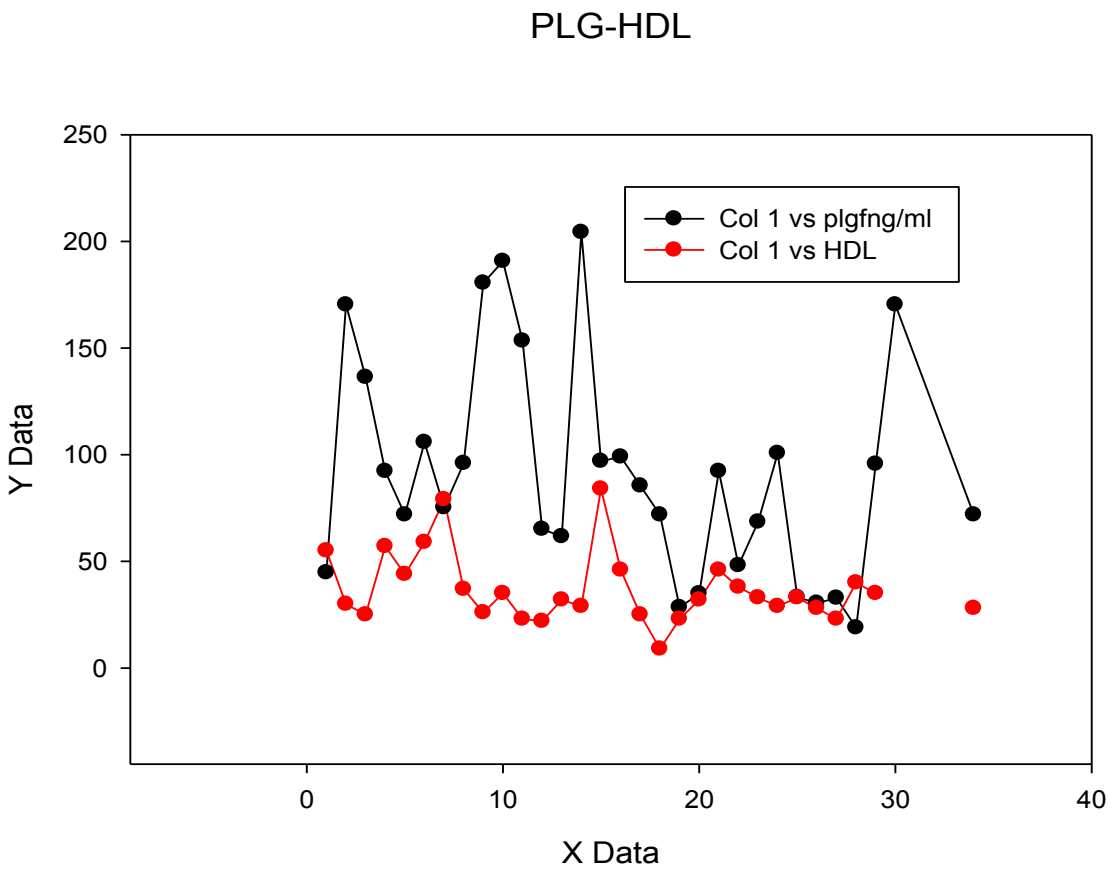

Figure 2: Inverse correlation between levels of PlGF and high density lipoprotein (HDL) in catheterized patients.

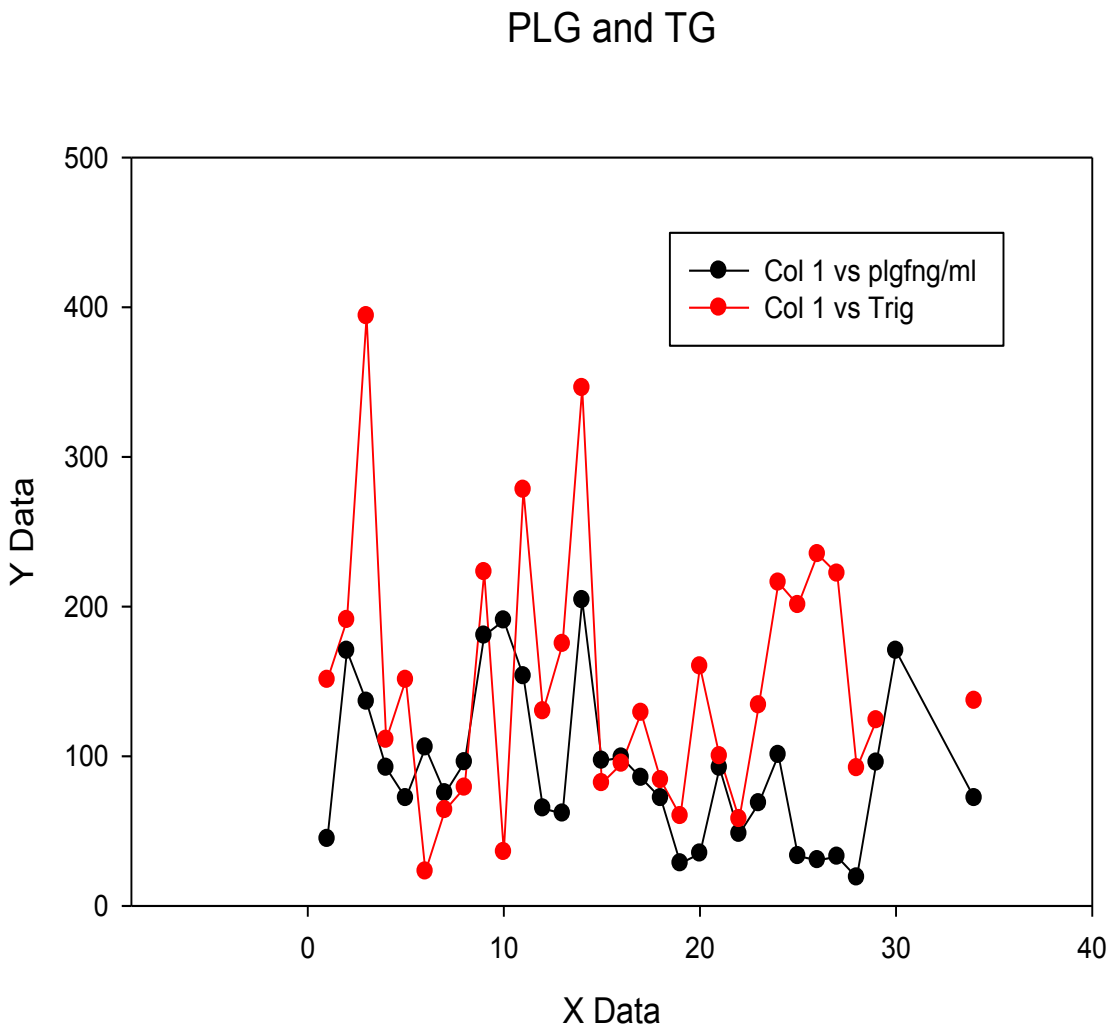

Figure 3: Direct correlation between levels of PlGF and triglycerides in catheterized patients.

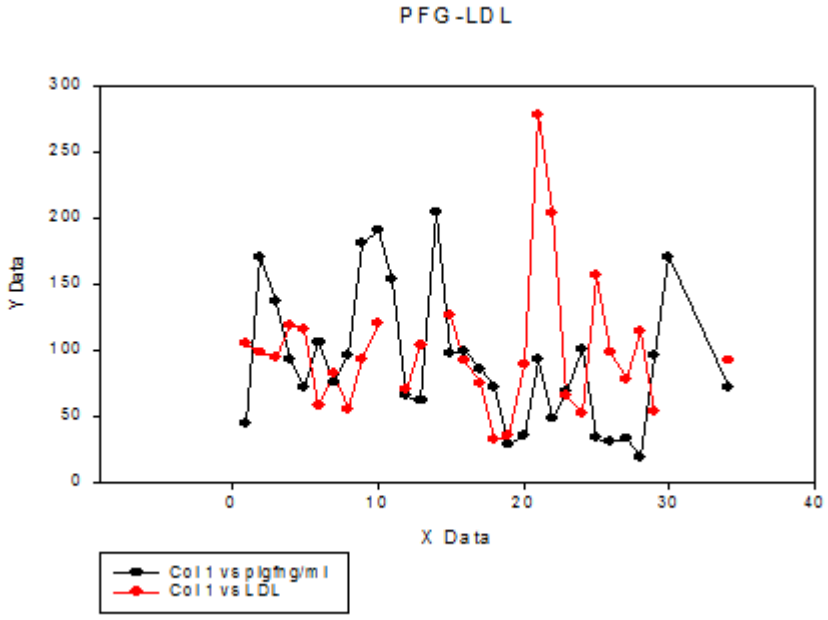

Figure 4: Lack of correlation between levels of PlGf and low density lipoprotein (LDL) in catheterized patients.